

AN EQUINE PROTOZOAL MYELOENCEPHALITIS CHALLENGE MODEL TESTING A SECOND TRANSPORT AFTER INOCULATION WITH *SARCOCYSTIS NEURONA* SPOROCCYSTS

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ABSTRACT: Previous challenge studies performed at Ohio State University involved a transport-stress model where the study animals were dosed with *Sarcocystis neurona* sporocysts on the day of arrival. This study was to test a second transportation of horses after oral inoculation with *S. neurona* sporocysts. Horses were assigned randomly to groups: group 1, transported 4 days after inoculation (DAI); group 2, at 11 DAI; group 3, at 18 DAI; and group 4, horses were not transported a second time (controls). An overall neurologic score was determined on the basis of a standard numbering system used by veterinarians. All scores are out of 5, which is the most severely affected animal. The mean score for the group 1 horses was 2.42; group 2 horses was 2.5; group 3 horses was 2.75; and group 4 horses was 3.25. Because the group 4 horses did not have a second transport, they were compared with all other groups. Statistically different scores were present between group 4 and groups 1 and 2. There was no difference in the time of seroconversion between groups. There was a difference between the time of onset of first clinical signs between groups 1 and 4. This difference was likely because of the different examination days. Differences in housing and handling were likely the reason for the differences in severity of clinical signs. This model results in consistent, significant clinical signs in all horses at approximately the same time period after inoculation but was most severe in horses that did not experience a second transport.

Equine protozoal myeloencephalitis (EPM) is 1 of the most important neurologic diseases in horses, reported to affect 14 of 10,000 horses per year (Dubey, Lindsay et al., 2001; NAHMS, 2001). This high case incidence emphasizes the importance of developing experimental models that will allow us to dissect the pathogenesis of disease. Although attributed primarily to *Sarcocystis neurona* infection, EPM has been difficult to reproduce in an experimental setting, raising questions as to the importance of protozoal challenge dose and the role of host factors such as stress in the induction of disease.

EPM is difficult to induce experimentally in horses, and Sofaly et al. (2002) recently summarized all attempts to do so by the various research groups. During the past 2 yr, extensive work has been reported regarding the life cycle of *S. neurona*, allowing us to overcome some of these limitations. Now, sporocysts of known origin and of defined species can be isolated, and the infectivity verified on the basis of the ability of preparations to infect interferon gamma gene knockout (KO) mice (Dubey et al., 2000; Cheadle, Tanhauser et al., 2001; Cheadle, Yowell et al., 2001; Dubey, Rosypal, Rosenthal et al., 2001; Dubey, Saville, Stanek et al., 2001).

Initial challenge studies in horses resulted in minimal to moderate clinical disease in affected horses, unlike naturally occurring cases of EPM that often cause severe neurologic deficits (Sofaly et al., 2002). Using sporocysts from the opossum–raccoon cycle, a recent report (the 2002 study) tested the hypothesis that different doses of *S. neurona* sporocysts would pro-

duce variations in seroconversion and clinical neurologic deficits in horses (Sofaly et al., 2002). Doses ranged from 1×10^2 to 1×10^6 sporocysts administered by nasogastric tube (Sofaly et al., 2002). As the sporocyst dose increased, time to seroconversion decreased and consistency of clinical neurologic signs improved (Sofaly et al., 2002). Another phenomenon that occurred during the 2002 study was worsening of clinical neurologic deficits after a short transport (27 km) to the Veterinary Teaching Hospital (W. J. A. Saville, pers. obs.). This worsening of clinical signs is the foundation for the hypothesis tested in the present work. Horses that are initially inoculated with *S. neurona* sporocysts after a first transportation and are transported at intervals after inoculation will develop more severe neurologic deficits than a group that was inoculated but not moved for a second time.

MATERIALS AND METHODS

Collection of *Sarcocystis neurona* sporocysts and preparation of horse inoculum

Sarcocystis neurona sporocysts were obtained from laboratory-raised opossums fed tissues of raccoons that were fed SN 37-R sporocysts as described (Sofaly et al., 2002; Stanek et al., 2002). Sporocysts were stored at 4 C for 14 days from the time of collection from opossums to feeding the horses. Sporocysts were bioassayed through interferon gamma gene KO mice to ensure viability as reported previously (Saville et al., 2001).

Experimental infection of horses

Draft and draft-crossed foals from an area of Saskatchewan, Canada, were screened for antibodies to *S. neurona*. A total of 26 foals that were negative for *S. neurona* antibody and were clinically normal were shipped to Fort Dodge Animal Health, Fort Dodge, Iowa (FDAH). These foals were 4- to 5-mo old. On arrival at the study site at Fort Dodge, all horses were examined physically and neurologically by a blinded observer. Samples collected after the neurologic examination included blood, using serum separator tubes, and whole blood for complete blood count using ethylenediaminetetraacetic acid as the anticoagulant. After sedation (Sedazine® and Torbugesic®; FDAH, Fort Dodge, Iowa), cerebrospinal fluid (CSF) was collected from each horse through the lumbosacral space as reported previously (Green et al.,

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TABLE I. Study groups.

Group no.	Horse no.	Date of second transport
1	2, 4, 15, 18, 22, 26	4 DAI
2	1, 5, 6, 9, 10, 17	11 DAI
3	3, 11, 23, 24, 27, 28	18 DAI
4	7, 8, 12, 13, 14, 20	NST*

* No second transport.

1992). After CSF collection, all horses were inoculated with 1.5×10^6 *S. neurona* sporocysts by nasogastric intubation.

The horses were randomly assigned to 1 of 4 study groups (Table I) on the day of arrival at Fort Dodge (hereinafter called day 0) (approximately 1,700 km). Horses from 3 groups were transported on either 4, 11, or 18 days after inoculation (DAI) to the study site at Finley farm of the Ohio State University (OSU) at West Jefferson, Ohio (approximately 1,200 km). Blinded neurologic examinations were performed on Saturdays at OSU and on Mondays at the FDAH facility by the same masked examiner.

At the FDAH facility, horses were housed in an isolation barn with concrete floors and rubber mats for bedding. They were fed native hay and oats grown locally. Water was provided ad libitum. The stalls in the isolation barn were open steel panels. Horses housed at the OSU facility were bedded on shavings on dirt floors and fed locally grown hay and provided water ad libitum. The stalls at OSU were wooden stalls with dirt floors. All horses were examined daily and findings were recorded by the persons at each facility. Weekly serum samples were collected at each facility on the same day that the blinded neurologic examinations were performed. At FDAH, examinations were performed on 7, 14, 21, and 28 DAI, and at OSU, examinations were performed on 5, 12, 19, 26, 33, and 40 DAI. All groups remained in the study for 4 wk, once they arrived at their final destination. After the 4-wk interval, horses were killed and complete postmortems performed.

Postmortem examination of horses

Central nervous tissues, i.e., brain and spinal cord, were fixed in 10% neutral buffered formalin for 10 days at room temperature before processing. At that time, the brain and spinal cord were sectioned at 1-cm intervals to examine for gross lesions. Representative sections were paraffin embedded, and 7 μ m sections stained with hematoxylin and eosin for light microscopic examination. These sections included: thalamus, mesencephalon, metencephalon including cerebellum, myelencephalon including the level of the obex, and sections of spinal cord taken at 9 different levels. Spinal-cord sections were examined both in cross-sectional and longitudinal planes at C₁₋₂, C₃₋₄, C₅₋₆, C_{7-T₁}, T₄₋₅, T₈₋₉, T₁₂₋₁₃, L₁₋₂, L₃₋₄ and L_{6-S₂}. The same technical person performed all sectioning. Tissue samples containing inflammatory changes were further processed for immunohistochemical staining of *S. neurona* antigen using a rabbit polyclonal antibody (Dubey and Hamir, 2000).

Statistical analysis

All horses were examined by a masked examiner who recorded a grade for each limb from 0–5 of 5 for weakness, spasticity, and ataxia. An overall scoring system to establish a neurologic grade for each horse was determined on the basis of the addition of points scored for each limb. This scoring system was determined on the basis of the distribution of scores for all horses at each time point. Horses that scored 1–3 points had an overall score of 0.5 of 5; horses that scored 4–6 points had an overall score of 1 of 5; horses that scored 7–9 points had an overall score of 1.5 of 5; horses that scored 10–12 points had an overall score of 2 of 5; horses that scored 13–15 points had an overall score of 2.5 of 5; horses that scored 16–18 points had an overall score of 3 of 5; and horses that scored 19–21 points had an overall score of 3.5 of 5. The overall scores for each horse were determined by each group, and using the group of horses that did not have a second transport as a control group, the mean scores were compared by group using Wilcoxon rank sum test (SAS Institute, Inc, Cary, North Carolina, version

8.2) with a significance level of $P < 0.05$. In addition, the Wilcoxon rank sum test was used to establish the significance of any differences in seroconversion times between groups and the time required for the initial detection of clinical signs.

RESULTS

Experimental horse results

Physical examination of horses: On the day of arrival at FDAH (Day 0), some horses had mucoid nasal and ocular discharge and 1 was febrile, otherwise, the physical examinations were considered normal. Neurologic examination of all horses did not detect any neurologic deficits on day 0, as well. Two horses (nos. 13 and 20) in group 4 developed high fever and clinical signs of a respiratory infection, requiring medical intervention during the study. Both horses were treated with a third-generation cephalosporin Naxcel® (Pharmacia, Kalamazoo, Michigan). Horse no. 20 experienced 2 episodes of elevated temperature and at postmortem had a mural abscess in the ventral colon. Horse no. 24 at OSU also developed a high fever with a mucopurulent nasal discharge, cough, and increased respiratory signs during the study. The horse was treated with procaine penicillin for 4 days and recovered. Transient fevers were noted periodically in all groups of horses, some shortly after transport (2–8 days) and some around 3 wk after challenge. Nine of 24 horses did not develop any increase in rectal temperature during the periods that they were being monitored. Except for the 3 horses mentioned previously that had received antibiotics, none of the rest of these horses received any medication. No further investigation was attempted to determine the etiology of these elevated temperatures.

Neurologic examination and serology: Group 1 (horses transported at 4 DAI)—All 6 horses developed readily detectable neurologic deficits ranging in severity from 1.5 to 3 of 5 (mean = 2.42). Detection of the first neurologic signs was at 5 DAI for 5 of the 6 animals and 12 DAI for the remaining horse. Seroconversion occurred in 3 horses at 12 DAI and 3 at 19 DAI. All horses had detectable antibodies to *S. neurona* in CSF when tested just before being killed (Table II).

Group 2 (horses transported at 11 DAI)—In this group, all 6 horses developed neurologic deficits ranging from 2 to 3 of 5 (mean = 2.5) in severity. Neurologic deficits were first detected in all 6 horses at 7 DAI. Five of 6 horses seroconverted at 19 DAI with 1 horse seroconverting at 12 DAI. Detectable antibodies to *S. neurona* were found in CSF of all 6 horses before euthanasia (Table II).

Group 3 (horses transported at 18 DAI)—Five of 6 horses had detectable neurologic deficits at 7 DAI, and 1 horse did not demonstrate deficits until 14 DAI. The range in severity of deficits for this group was between 2 and 3.5 of 5 (mean = 2.7). Three of 6 horses seroconverted at 14 DAI, and 3 seroconverted at 19 DAI with positive antibodies to *S. neurona* in CSF of all 6 horses at the time of being killed (Table II).

Group 4 (horses that did not undergo a second transport)—All 6 horses in this group had detectable neurologic deficits at 7 DAI. The range in severity of the deficits was between 3 and 3.5 of 5 (mean = 3.25). Three of 6 horses seroconverted at 14 DAI and 3 at 21 DAI. All 6 horses had detectable antibodies to *S. neurona* in the CSF at the time of euthanasia (Table II).

Postmortem examination: Sporadic axonal degenerative changes were consistently identified within the spinal cord

TABLE II. Summary table by group and by horse number for sex, date of seroconversion, antibodies detected in CSF ending neurologic examination score, and days of first detection of clinical signs.

Group no.	Horse no.	Sex	Seroconversion (DAI)	CSF*	Ending <i>Sarcocystis neurona</i> sporocysts neurologic examination score (masked)	DFS†
1	2	Male	12	Positive	2	5
	4	Male	19	Positive	1.5	12
	15	Male	19	Positive	3	5
	18	Male	12	Positive	3	5
	22	Male	12	Positive	2.5	5
	26	Male	19	Positive	2.5	5
2	1	Male	19	Positive	3	7
	5	Female	19	Positive	2	7
	6	Female	12	Positive	2.5	7
	9	Female	19	Positive	2.5	7
	10	Male	19	Positive	2.5	7
	17	Male	19	Positive	2.5	7
3	3	Male	14	Positive	3	7
	11	Male	19	Positive	3	7
	23	Male	14	Positive	2.5	7
	24	Female	14	Positive	3.5	7
	27	Female	19	Positive	2	7
	28	Female	19	Positive	2.5	14
4	7	Male	21	Positive	3	7
	8	Female	14	Positive	3.5	7
	12	Male	14	Positive	3	7
	13	Male	21	Positive	3	7
	14	Female	21	Positive	3.5	7
	20	Male	14	Positive	3.5	7

* CSF antibody to *S. neurona*.

† DFS = Day of first detection of neurologic signs after inoculation.

white matter of all horses examined. These changes were interpreted as incidental findings appropriate to the age of animal and cannot be linked to the *S. neurona* challenge. Single foci of perivascular infiltrates were not considered important findings; although such changes can be caused by *S. neurona* challenge, they are considered nonspecific. Multiple foci of leptomeningeal and parenchymal perivascular lymphohistiocytic infiltrates distributed randomly among either gray or white matter were considered significant, regardless of the magnitude and were interpreted as compatible with a response to *S. neurona* challenge. However, the tissue change most consistent with a protozoal etiology consisted of glial nodules formed in parenchyma associated with the lymphohistiocytic perivascular cuffs. Accordingly, horses were grouped into 1 of 3 categories as follows: no significant microscopic lesion (horses nos. 2, 3, 4, 7, 8, 11, 12, 14, 15, 17, 18, 26), including 4 animals that exhibited single inflammatory foci (horses nos. 2, 14, 15, 17); minimal but significant inflammatory changes (horses nos. 1, 5, 6, 9, 10, 13, 22, 24, 27, 28); significant inflammatory changes inclusive of glial parenchymal responses that are suggestive of a protozoal etiology (horses nos. 20 and 23). Immunohistochemical staining for *S. neurona* antigen was performed on tissue sections exhibiting the most severe lesions (horses nos. 13, 20, and 23). There was no *S. neurona* antigen detected in any of the tissues examined.

Histological changes were observed in animals representative of all treatment groups. Group 1 (horses transported at 4

DAI)—Only 1 horse (no. 22) from this group had significant although minimal inflammatory changes in the brain and spinal cord. These changes consisted of lymphohistiocytic perivascular infiltrates involving the parenchyma or meninges (or both) at multiple spinal cord levels. The other 5 horses either had no significant microscopic lesions (4 of 5) or only single inflammatory foci (1 of 5) within the brain or spinal cord (or both).

Group 2 (horses transported at 11 DAI)—Five of 6 horses in this group had minimal inflammatory changes compatible with response to an infectious etiology such as *S. neurona*, and 1 horse (no. 17) had a single inflammatory foci.

Group 3 (horses transported at 18 DAI)—Minimal inflammatory lesions were detected in 3 of 6 horses in this group, with lesions in a fourth horse being increased in severity (i.e., mild) (no. 23). The latter also exhibited axonal spheroids in nuclei at the level of the obex, suggestive of equine degenerative myeloencephalopathy. Two horses did not have any significant lesions that would support changes induced by *S. neurona*. One horse (no. 24) exhibited axonal degenerative changes in white matter funiculi of the spinal cord that were suggestive of focal vascular compromise.

Group 4 (horses that did not undergo a second transport)—Histologic examination revealed significant inflammatory lesions suggestive of *S. neurona* in 2 of 6 horses in this group. Lesions in these animals consisted not only of lymphohistiocytic perivascular infiltrates but also of glial nodule formation in parenchyma adjacent to the inflammatory infiltrates. Two of

6 horses had single inflammatory foci and the other 2 horses had no significant lesion after microscopic evaluation of central nervous tissues.

Statistical analysis

Because the horses in group 4 were not subjected to a second stressful event (transport), this group was used as the comparison group for the statistical analysis. When comparing the neurologic grade score between groups 4 and 1, they were significantly different ($P = 0.0119$). Significant differences were also detected when comparing groups 4 and 2 ($P = 0.0058$). When comparing group 4 with group 3, although not statistically different, there was a trend ($P = 0.075$). Day of first detection of antibody was tested using group 4 as the comparison group. No statistical difference was detected between groups. When testing differences between days of first clinical neurologic deficits detected, there was no difference between group 4 horses and groups 2 and 3. There was a difference between groups 1 and 4 ($P = 0.0325$).

Bioassay in mice

A 1×10^{-5} dilution of the inoculum fed to each horse was lethal to KO mice. All KO mice (5 per dilution) fed sporocysts up to 1×10^{-5} dilution developed neurologic signs, and *S. neurona* was demonstrable in their tissues. The KO mice fed 1×10^{-6} to 1×10^{-9} dilutions remained healthy, and neither parasites nor *S. neurona* antibodies was demonstrable in them. Thus, each horse received at least 100,000 mouse infective doses of *S. neurona* sporocysts.

DISCUSSION

In this study, the hypothesis tested was the effect of a second transport after sporocyst challenge on the day of arrival from the first transport. This study was able to detect statistically different grades of neurologic disease in horses transported for a second time compared with horses that were not transported for a second time. The hypothesis was based on observations in previous studies at OSU, where horses that were transported a second time after challenge developed a worsening of the neurologic deficits in 60% of the horses examined (W. J. A. Saville, pers. obs.). The intent was to investigate this phenomenon in a controlled fashion to determine whether more severe neurologic signs could be elicited compared with horses managed as in previous studies. Interestingly, the groups of horses that were transported for a second time after challenge had lower mean neurologic grades than the group of horses that remained at FDAH.

All horses seroconverted between 12 and 21 DAI. This result is similar to previous reports that detected antibody at 14 DAI in transport-stressed horses receiving sporocysts on the day of arrival (Saville et al., 2001; Sofaly et al., 2002). This study demonstrated somewhat longer seroconversion times with some horses, but it was likely a function of timing of sample collection. All samples were collected at the time of the weekly neurologic exams, and because the 2 study sites were 1,200 km apart, and the examiner was the same person for both sites, same day sampling was not possible. Because of the weekly sampling scheme, there is no means of knowing whether the

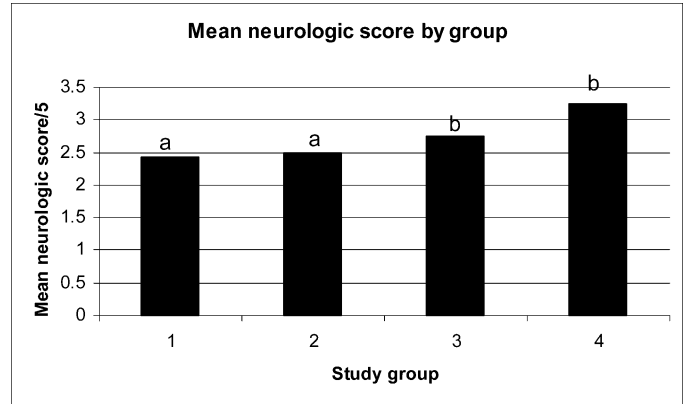


FIGURE 1. This graph depicts the mean neurologic score by group of horses. Group 4 horses were statistically different from groups 1 and 2.

horses seroconverted on days 13 through 18 in the OSU study animals or days 14 through 20 in the FDAH study animals.

The earliest time that detectable neurologic deficits were seen was 5 to 7 DAI in 22 of 24 horses. This is earlier than that in previous reports, regardless of whether the horses were transported once or twice. In a previous report, horses that received 1×10^6 sporocysts demonstrated clinical signs at 8 DAI (3 of 4) and 22 DAI in 1 horse (Sofaly et al., 2002). In that same study, a dose-response relationship was noted (Sofaly et al., 2002). Although not likely different, this may also be related to dose of the parasite because horses in the present study were inoculated with 1.5×10^6 sporocysts. The differences detected between the group 1 and group 4 horses may have been related to the second transport; however, the timing of the neurologic examinations was different in this study compared with the previous study, which may also account for some differences.

The horses that were not subjected to a second transport exhibited the most severe neurologic signs compared with groups that did undergo a second transport. This is in contrast to findings from our previous studies. There were obvious differences in housing between FDAH and OSU. At FDAH, the horses were housed in steel-paneled pens with openings between stalls, whereas, the horses at OSU were stalled in wooden structures with no communication possible between the horses. All neurologic examinations were carried out in the building at FDAH, whereas, at OSU, all neurologic examinations were conducted in the aisle and outside. These were conducted outside because of space constraints in the OSU barn. The horses at FDAH were able to view procedures on other horses, and there was no sound proofing in their building, which may have influenced the stress in that group of horses. Another issue was the personnel handling the horses. At FDAH, different personnel handled the horses on a daily or weekly basis, whereas, the same personnel handled all horses on a daily basis at OSU. Familiarity with their handlers may have influenced the level of stress on the study animals at OSU. Whether the examinations were inside or outside or the housing or handling was different, the investigators expected the second transport would have resulted in more severe neurologic deficits because of the immunosuppressive effects of transport.

As has happened in all previous challenge model studies,

parasite has not been detected on postmortem (Fenger et al., 1997; Cutler et al., 2001; Saville et al., 2001; Sofaly et al., 2002). However, the authors believe this is likely a function of timing of necropsy. Previous inoculation of horses with 1×10^8 opossum- or raccoon-derived sporocysts with necropsy at days 7 and 14 postinoculation resulted in negative findings as well, suggesting that the parasite may be cleared very quickly, possibly earlier than 7 days (W. J. A. Saville and J. P. Dubey, pers. obs.). This further supports the hypothesis that clinical signs of EPM may be a result of cytokine mediators more than direct nerve cell body infection as discussed in a previous study (Sofaly et al., 2002). In 1 study where a severe combined immunodeficient foal was infected with *S. neurona* sporocysts, parasite DNA was detected in the central nervous system (CNS) (Long et al., 2002). However, this foal neither developed neurologic deficits nor was there lesions in the CNS suggestive of *S. neurona* infection (Long et al., 2002). Because there was no deficit and no lesion, this would suggest that, in horses, an intact immune system may be required for the parasite to cause EPM.

Although the hypothesis was refuted that a second transport would lead to more severe neurologic signs, there are several positive findings in this study. The severity of the clinical neurologic deficits was consistent for all groups (range 2.42–3.25 of 5). Basically, all foals were moderately to severely affected compared with previous studies, where many were mildly affected. In addition, the onset of first clinical signs and time to seroconversion were consistent between groups. This challenge model, using transport stress and raccoon-opossum cycle-derived sporocysts, consistently produces readily detectable neurologic deficits with a 1-time dose of 1.5×10^6 sporocysts. Therefore, this challenge model could be used to investigate the efficacy of preventive medications or vaccines to test their ability to prevent development of clinical signs of EPM.

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LITERATURE CITED

- CHEADLE, M. A., S. M. TANHAUSER, J. B. DAME, D. C. SELLON, M. HINES, P. E. GINN, R. J. MACKAY, AND E. C. GREINER. 2001. The nine-banded armadillo (*Dasypus novemcinctus*) is an intermediate host for *Sarcocystis neurona*. *International Journal for Parasitology* **31**: 330–335.
- , C. A. YOWELL, D. C. SELLON, M. HINES, P. E. GINN, A. E. MARSH, J. B. DAME, AND ———. 2001. The striped skunk (*Mephitis mephitis*) is an intermediate host for *Sarcocystis neurona*. *International Journal for Parasitology* **31**: 843–849.
- CUTLER, T., R. MACKAY, P. E. GINN, K. GILLIS, S. M. TANHAUSER, E. V. LERAY, J. B. DAME, AND E. C. GREINER. 2001. Immunoreconversion against *Sarcocystis neurona* in normal and dexamethasone-treated horses challenged with *S. neurona* sporocysts. *Veterinary Parasitology* **95**: 197–210.
- DUBEY, J. P., AND A. HAMIR. 2000. Immunohistochemical confirmation of *Sarcocystis neurona* infections in raccoons, mink, cat, skunk, and pony. *Journal of Parasitology* **86**: 1150–1152.
- , AND D. S. LINDSAY. 1998. Isolation in immunodeficient mice of *Sarcocystis neurona* from opossum (*Didelphis virginiana*) faeces, and its differentiation from *Sarcocystis falcatula*. *International Journal for Parasitology* **28**: 1823–1828.
- , W. J. A. SAVILLE, S. M. REED, D. E. GRANSTROM, AND C. A. SPEER. 2001. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). *Veterinary Parasitology* **95**: 89–131.
- , A. C. ROSYPAL, B. M. ROSENTHAL, N. J. THOMAS, D. S. LINDSAY, J. F. STANEK, S. M. REED, AND W. J. A. SAVILLE. 2001. *Sarcocystis neurona* infections in sea otter (*Enhydra lutri*): Evidence for natural infections with sarcocysts and transmission of infection to opossums (*Didelphis virginiana*). *Journal of Parasitology* **87**: 1387–1393.
- , W. J. A. SAVILLE, D. S. LINDSAY, R. W. STICH, J. F. STANEK, C. A. SPEER, B. M. ROSENTHAL, C. J. NJOKU, O. C. H. KWOK, S. K. SHEN, AND S. M. REED. 2000. Completion of the life cycle of *Sarcocystis neurona*. *Journal of Parasitology* **86**: 1276–1280.
- , J. F. STANEK, D. S. LINDSAY, B. M. ROSENTHAL, M. J. OGLESBEE, A. C. ROSYPAL, C. J. NJOKU, R. W. STICH, O. C. H. KWOK, S. K. SHEN, A. N. HAMIR, AND S. M. REED. 2001. *Sarcocystis neurona* infections in raccoons (*Procyon lotor*): Evidence for natural infection with sarcocysts, transmission of infection to opossums (*Didelphis virginiana*) and experimental induction of neurologic disease in raccoons. *Veterinary Parasitology* **100**: 117–129.
- FENDER, C. K., D. E. GRANSTROM, A. A. GAJADHAR, N. M. WILLIAMS, S. A. MCCRILLIS, S. STAMPER, J. L. LANGEMEIER, AND J. P. DUBEY. 1997. Experimental induction of equine protozoal myeloencephalitis in horses using *Sarcocystis* sp. sporocysts from the opossum (*Didelphis virginiana*). *Veterinary Parasitology* **68**: 199–213.
- GREEN, E., G. M. CONSTANTINESCU, AND R. A. KROLL. 1992. Equine cerebrospinal fluid: Physiologic principles and collection techniques. *Compendium for Continuing Education of Practicing Veterinarians* **14**: 229–237.
- LONG, M. T., M. T. HINES, D. P. KNOWLES, S. TANHAUSER, J. B. DAME, T. J. CUTLER, R. J. MACKAY, AND D. C. SELLON. 2002. *Sarcocystis neurona*: Parasitemia in a severe combined immune deficient (SCID) horse fed sporocysts. *Experimental Parasitology* **100**: 150–154.
- NAHMS. 2001. Equine protozoal myeloencephalitis (EPM) in the U.S. #312.0401. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System, Fort Collins, Colorado.
- SAVILLE, W. J. A., R. W. STICH, S. M. REED, C. J. NJOKU, M. J. OGLESBEE, A. WUNSCHMANN, D. L. GROVER, A. L. LAREW-NAUGLE, J. F. STANEK, D. E. GRANSTROM, AND J. P. DUBEY. 2001. Utilization of stress in the development of an equine model for equine protozoal myeloencephalitis. *Veterinary Parasitology* **95**: 211–222.
- SOFAFY, C. D., S. M. REED, J. C. GORDON, J. P. DUBEY, M. J. OGLESBEE, C. J. NJOKU, D. L. GROVER, AND W. J. A. SAVILLE. 2002. Experimental induction of equine protozoal myeloencephalitis (EPM) in the horse: Effect of *Sarcocystis neurona* sporocyst inoculation on the development of clinical neurologic disease. *Journal of Parasitology* **88**: 1164–1170.
- STANEK, J. F., J. P. DUBEY, M. J. OGLESBEE, S. M. REED, D. S. LINDSAY, C. J. NJOKU, AND W. J. A. SAVILLE. 2002. Life cycle of *Sarcocystis neurona* infections in its natural intermediate host, raccoon (*Procyon lotor*). *Journal of Parasitology* **88**: 1151–1158.